HHS Public Access

Author manuscript

Annu Rev Immunol. Author manuscript; available in PMC 2015 July 06.

Published in final edited form as:

Annu Rev Immunol. 2015 ; 33: 355–391. doi:10.1146/annurev-immunol-032414-112103.

TAM Receptor Signaling in Immune Homeostasis

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Abstract

The TAM receptor tyrosine kinases (RTKs)—TYRO3, AXL, and MERTK—together with their cognate agonists GAS6 and PROS1 play an essential role in the resolution of inflammation. Deficiencies in TAM signaling have been associated with chronic inflammatory and autoimmune diseases. Three processes regulated by TAM signaling may contribute, either independently or collectively, to immune homeostasis: the negative regulation of the innate immune response, the phagocytosis of apoptotic cells, and the restoration of vascular integrity. Recent studies have also revealed the function of TAMs in infectious diseases and cancer. Here, we review the important milestones in the discovery of these RTKs and their ligands and the studies that underscore the functional importance of this signaling pathway in physiological immune settings and disease.

Keywords

TYRO3; AXL; MERTK; GAS6; PROS1

INTRODUCTION

In the epic *Mahabharata*, on the thirteenth day of the great war, the valiant warrior Abhimanyu used his exclusive knowledge of military tactics to penetrate a purportedly invincible enemy formation termed *Chakravyuha* (a troop array that resembles a wheel). The outcome of this battle, alas, was preordained in the divine scheme of *dharma*. Despite his remarkable bravery, skills, and courage, Abhimanyu's demise was inevitable, for he knew only how to enter the formation and not how to exit. Sans an exit strategy, he was fated to become a tragic hero in this tale.

DISCLOSURE STATEMENT

C.V.R. is a cofounder of Xetrios Therapeutics and is an inventor on patents for targeting TAM RTKs in the regulation of the inflammatory response.

NOTE ADDED IN PROOF

These are exciting times in TAM research. Many new discoveries have been reported after this manuscript was accepted for publication. Zagórska and colleagues (198) reported diversification of function among TAM RTKs in the context of inflammatory and noninflammatory phagocytosis of apoptotic cells. Lew and colleagues (199) and Tsou and colleagues (200) described full-length TAM receptor affinities for the ligands in the presence of PtdSer. A recent review by Graham and colleagues (201) summarizes many exciting developments in TAM-targeted cancer therapy.

Similar are the rules for most biological processes: Engagement is followed by a return to baseline, and therefore homeostasis requires an exit strategy. For example, the immune response to an infection can be initiated with the recognition of the pathogen by the innate immune system (1). Subsequently, the immune system combats the invading pathogen by deploying a wide array of molecular weapons. Although the machineries of pathogen recognition and clearance are its ostensible components, a physiological immune response also involves negative regulatory mechanisms that provide a variety of safeguards and exit strategies. These mechanisms do not directly function in pathogen sensing or clearance, but in their absence even the most successful immune response might prove just as fatal to the host as the inciting infection.

Its essential function in protecting the organism notwithstanding, the immune response carries a risk of collateral damage and even autoimmunity. Avoiding an immune response against self through the distinction of self versus nonself is equally a measure of a healthy immune system, as is the robustness of the antipathogen response. This distinction is achieved by various mechanisms, such as selective recognition of nonself patterns by cells of the innate immune system (1) and central tolerance mechanisms in adaptive immunity including clonal deletion, editing, and anergy (2–5). Although these are mechanisms that prevent illegitimate engagement, even a bona fide immune response can cause havoc if it is not adequately tailored to the pathogen or if it is overzealous. Therefore, specialized subsets of immune cells are deployed to ensure a selective reaction. $CD4^+$ helper T cell subtypes (Th1, Th2, Th17, Tfh) and $CD8^+$ T cells represent this form of specialization. Furthermore, T regulatory cells (Tregs) can regulate the nature and intensity of the immune response and thus represent a key component of peripheral tolerance. In the absence of Tregs, both illegitimate (autoimmunity) and exaggerated responses to nonself can occur (6). Additional safeguards also exist to ensure appropriate intensity and duration of the immune response. Negative regulation through molecules such as cytotoxic T lymphocyte–associated protein (CTLA)-4 and programmed death (PD)-1, contraction of the response, and activation-induced cell death (AICD) of lymphocytes are all mechanisms to ensure an optimal level of immune response and eventual withdrawal (7–10). Lastly, once the pathogen has been cleared, a series of steps ensure the resolution of the inflammatory response and the restoration of tissue homeostasis (11). Systems that sense resolution are coupled to removal of debris and to tissue repair to regain tissue/organ function.

In this review, we describe a family of receptor tyrosine kinases (RTKs) and their ligands that are essential for the homeostasis of the immune response. This signaling system not only controls the magnitude of the immune response by dampening inflammation, but also likely integrates the phasing out of the innate immune response with active processes that favor the resolution of inflammation and recovery of tissue function via the clearance of apoptotic debris and the restoration of vascular integrity. Over two decades of remarkable discoveries notwithstanding, the TAM RTKs and their signaling functions continue to reveal incredible insights into the biology of the immune response and promise novel therapeutic avenues that may improve the outcome in a gamut of diseases as diverse as infection, chronic inflammatory and autoimmune conditions, and cancer.

IDENTIFICATION AND CLONING

Hunter and colleagues' (12) discovery of reversible tyrosine phosphorylation and its pivotal role in cellular transformation catalyzed a concerted attempt to identify members of the RTK family in the 1980s. Many newly discovered RTKs were revealed to have important roles in cellular differentiation during development. As part of an effort to characterize the repertoire of RTKs that may function in differentiation and development of the peripheral nervous system, Lai & Lemke (13) identified 13 novel genes based on homology with RTK domains from a Schwann cell library. Three of these genes—*Tyro3*, *Tyro7*, and *Tyro12*—were clustered as a unique subgroup based on sequence identity. The computational classification of the kinome into a hierarchy of groups, families, and subfamilies in 2002 preserves the original clustering of these three RTKs as a distinct subfamily (14).

The full-length cDNAs for *Tyro3*, *Tyro7*, and *Tyro12* were independently cloned in many different laboratories. The cloning of full-length *Tyro3* was first described by Crosier et al. (15), who named it *Dtk* for developmental tyrosine kinase based on its expression during the differentiation of murine stem cells. Two months later, three back-to-back papers (16–18) reported the cloning of *Tyro3* and referred to it as *Brt* (brain tyrosine kinase), *SKY* (cloned based on homology to *vsea; sea*-related tyrosine kinase), and *TIF* (tyrosine kinase with immunoglobulin and fibronectin type III domains), respectively. Subsequently, Mark et al. (19) reported the cloning of *Rse* (*receptor sectatoris*) and Lai et al. (20) the cloning of *Tyro3*. Similarly, three different groups cloned full-length cDNAs of *Tyro7*. O'Bryan et al. (21) termed the gene *AXL* after the Greek word *anexelekto* meaning unchecked or uncontrolled, due the abnormal nature of cell growth in the presence of this gene. Janssen et al. (22) named the gene *Ufo* in oblique reference to its yet unidentified function. Finally, Rescigno et al. (23) called the same gene *Ark* (for adhesion-related kinase) based on its predicted function because it contains domains characteristic of neural cell adhesion molecules. *Tyro12* was cloned first as a viral oncogene, *v-Ryk*, and then as its cellular counterpart, *c-Eyk*, by Jia et al. (24, 25). The human gene was cloned by Graham et al. (26) and identified as *MER* due to its expression in monocytes and in epithelial and reproductive tissues. The diverse and disparate nomenclature for these genes has generated tremendous confusion in surveying the literature. Throughout the rest of this review, we will refer to *Tyro3/Dtk/Brt/Sky/Tif/Rse* as *Tyro3*, *Tyro7/Axl/Ufo/Ark* as *Axl*, and *Tyro12/v-Ryk/c-Eyk/Mer* as *Mertk*, which is the nomenclature provided by Mouse Genome Informatics and HUGO Gene Nomenclature Committee. When referring to TYRO3, AXL, and MERTK collectively, we will use the term TAM receptors or TAM RTKs.

For nearly half a decade after the TAM RTKs were cloned, the identity of the ligands that bind and activate them remained enigmatic. In 1995, PROS1 [Protein S, named after Seattle, the city where it was discovered (27)] was identified as an active component of conditioned media from the adult bovine aortic endothelial (ABAE) cell line that phosphorylated TYRO3 (28). PROS1 also induced TYRO3-dependent growth responses, indicating that PROS1 is an agonist of TYRO3. Human PROS1 was purified from plasma in 1977. Following the cloning of bovine *Pros1* (29), the human gene was cloned by Lundwall et al. (30) from a fetal liver phage λgt11 cDNA library by using DNA fragments from bovine *Pros1* and human *PROC* (Protein C). Interestingly, ABAE-derived PROS1 agonistic activity

was specific to TYRO3, whereas another ABAE-derived protein, GAS6 (growth-arrest-specific 6), functioned as an AXL agonist (28). *Gas6* was originally cloned from a NIH/3T3 subtraction cDNA library enriched for genes expressed under conditions of growth arrest induced by serum deprivation (31). We refer to PROS1 and GAS6 collectively as the TAM ligands or TAM agonists.

STRUCTURAL DETERMINANTS

TAM Ligands

Both PROS1 and GAS6 are Gla domain–containing proteins, i.e., proteins containing gamma-carboxylated glutamic acid residues. The gamma-carboxylation of glutamate residues vastly increases their ability to bind Ca^{2+}. The carboxylation reaction involves the abstraction of a proton from the 4-carbon of glutamate by reduced vitamin K. In this process, vitamin K is converted into vitamin K epoxide. Vitamin K epoxide reductase (VKOR) reconverts the vitamin K epoxide back into vitamin K. Therefore, gamma-carboxylation and the function of Gla domain–containing proteins can be affected by dietary and other sources of vitamin K and the exposure to chemicals such as warfarin that inhibit VKOR (32).

The Gla domain was originally identified in the blood coagulation factor prothrombin. Prothrombin undergoes gamma-carboxylation on ten glutamic acid residues. This enables prothrombin to bind, in a Ca^{2+}-dependent manner, a negatively charged phospholipid, phosphatidylserine (PtdSer), that is exposed on the surface of activated platelets. PtdSer is almost exclusively located on the inner leaflet of plasma membranes. A number of enzymatic activities control PtdSer localization in the cell (33). Flippases (P4-ATPases) translocate PtdSer to the inner leaflet of the plasma membrane, while ABC-type transporters (or floppases) move PtdSer to the outer layer. Both processes are dependent on ATP. A third class of enzymes known as scramblases translocate PtdSer bi-directionally in an ATP-independent manner. During platelet activation, flippases are inhibited, whereas the increase in intracellular calcium results in the activation of the scramblase TMEM16F, which results in the exposure of PtdSer on the outer leaflet of the platelet plasma membrane (34). In addition to prothrombin, many procoagulant proteins, such as Factors VII, IX, and X, also contain Gla domains and are, therefore, recruited to the platelet surface to form the clot.

Gla domains are also found in anticoagulant proteins. Similar to the inflammatory response, coagulation requires negative regulation so as to prevent pathological thrombosis. This is achieved, in part, by regulated anticoagulant pathways. In fact, the most well-characterized function of PROS1 is its TAM receptor–independent role as an anticoagulant (35, 36). PROS1 is found in the bloodstream at approximately 300 nM, where it functions as a cofactor for activated Protein C (aPC), another Gla domain–containing protein (37). In the coagulation cascade—a process that is essential for preventing exsanguination—rapid induction and amplification are achieved through a series of sequential protease activation reactions. The coagulation Factors V and VIII are required for prothrombin cleavage and clot formation. PROS1-aPC promotes the degradation of Factors V and VIII and prevents thrombin formation. PROS1 also has direct, aPC-independent, anticoagulant functions in inhibiting prothrombin cleavage (38). Gas6 does not have known aPC cofactor activity.

GAS6 and PROS1 contain Gla domains that are ∼50–amino acid stretches near their N termini. Gamma-carboxylation and PtdSer binding are essential for the maximal bioactivity of both full-length TAM ligands (39–42). For example, PROS1 cofactor activity for aPC and its ability to activate the TAM RTKs depend on gamma-carboxylation of the GLA domain and binding to PtdSer.

At the C termini of the two TAM ligands are laminin G (globular) domains. These domains are found in basement membrane proteins, such as laminin, agrin, perlecan, and collagen, and in the cell adhesion molecules neurexins. Additionally, the sex hormone binding globulin contains two laminin G domains closely related to that of TAM ligands.

Four epidermal growth factor (EGF)-like repeats lie between the N-terminal Gla domain and the C-terminal tandem laminin G domains of the TAM ligands. Named after the domain found in the EGF, this extracellular domain is conserved in EGF family members such as heregulin-α and TNF-α. Structurally, these EGF-like repeats consist of two β-sheets—the first sheet with three β-strands and the second shorter sheet with two β-strands—and they have six conserved cysteine residues forming three intradomain disulfide linkages. This is a common domain found in components of the coagulation cascade, such as Factors VII, IX, and X, Protein C, and thrombomodulin, as well as in many other proteins, including tenascin-C and fibrilin1.

PROS1 but not GAS6 contains a thrombin-sensitive region between the Gla domain and the first EGF-like repeat (43). Thrombin cleaves PROS1 at Arg^{49} and Arg^{70} to release a 21–amino acid peptide (43). Various other proteases including Factor Xa and elastase also cleave PROS1 in vitro. Factor Xa cleaves PROS1 at Arg^{60}, whereas elastase cleaves at Val^{73} (43). These proteolytic activities inhibit PROS1 cofactor function in vitro, but whether they occur in vivo and affect PROS1 ability to activate the TAM RTKs remains unknown.

Much like thrombin, which functions in coagulation as a serine protease and binds to and activates the thrombin-receptors (protease-activated receptors, or PARs), PROS1 has dual functions as both an anticoagulant and a ligand for TAM RTK. Unlike thrombin, which relies on its protease activity for both of its functions, distinct regions of PROS1 have been implicated in its anticoagulant and anti-inflammatory roles. The binding between PROS1 and aPC has been mapped to the N-terminal portion of PROS1. A single point mutation within the EGF1 domain (D95A) is sufficient to severely compromise PROS1's function as a cofactor of aPC in the proteolytic inactivation of Factor Va and Factor VIIIa (44). However, the C-terminal laminin G domains of PROS1 mediate the interaction with TAM RTKs and are sufficient to induce the activation of the receptors (45).

In humans, the laminin G domains of PROS1 have been shown to bind to the complement component C4b-binding protein (C4BP) (46). C4BP is composed of seven α-chains and one β-chain linked through disulfide bridges, and binding to PROS1 occurs through the β-chain. Approximately 60% of PROS1 in the bloodstream circulates bound to C4BP. PROS1 needs to be in its free form to function as a cofactor for aPC. As such, increases in the expression of C4BP, as detected during inflammation, limit the availability of free PROS1 and favor a procoagulant state. Binding of C4BP to the C-terminal region of PROS1 is also predicted to

compete with binding to TAM RTKs and has been shown to inhibit PROS1-mediated activation of TYRO3 in vitro (47). The biological implications of such competition are unknown. Surprisingly, two in-phase stop codons preclude expression of a functional C4BPβ in the mouse (48). Thus, murine C4BP lacks the β-chain and the capacity to bind PROS1. GAS6 plasma concentration is significantly lower than PROS1 and so is its affinity for C4BP.

TAM RTKs

The TAM RTKs also share structural similarities. From the extracellular N termini to the cytosolic C termini, these receptors display two Ig-like domains, two fibronectin type III (FNIII) domains, a hydrophobic transmembrane domain, and a tyrosine kinase domain. Ig-like domains are formed by seven to nine antiparallel β-strands. FNIII domains are all β-strand structures with similarities to Ig folds. This domain is ubiquitous, found in approximately 2% of all animal proteins, including cell adhesion molecules, cell surface hormone and cytokine receptors, chaperonins, and carbohydrate binding proteins.

The two Ig-like domains of TAM RTK are involved in interactions with the TAM ligands. The crystal structure of GAS6 laminin G domains/AXL Ig-like domains reveals a 2:2 symmetric GAS6/AXL assembly with two distinct ligand-receptor contacts (49). The major contact is between the first laminin G domain of GAS6 and the first Ig-like domain of AXL. An additional, minor contact between the first laminin G domain of GAS6 with the second Ig-like domain AXL was also reported. Mutagenesis experiments indicate that both interactions are essential for receptor activation (49). Therefore, only the first laminin G domain of TAM ligands is believed to participate in the receptor–ligand interaction. However, it is important to note that this prediction is based on a crystal structure derived from a fragment of the ligand that lacks the Gla domain and PtdSer interactions. Thus, it remains unclear how PtdSer binding alters the conformation of the TAM ligands and affects TAM RTK binding.

Between the FNIII domains and the transmembrane domain, Pro^{485} renders MERTK susceptible to cleavage by the metalloproteinase ADAM17 (50). Site-directed mutagenesis of this cleavage site results in MERTK resistance to proteolysis. Activation of pattern-recognition receptors with lipopolysaccharide (LPS) or PolyI:C in macrophages has been shown to induce cleavage of MERTK at this proline site and induce shedding of the MERTK extracellular domain. Furthermore, LPS- and PolyI:C-induced cleavage is dependent on ADAM17, as it is abrogated in *Adam17* knockout macrophages. A similar proteolytic cleavage has been described for AXL (51, 52). Investigators have suggested that such cleavage results in the inactivation of the receptor and neutralization of TAM ligands by the released ectodomain of the receptors (50, 53). Engineering the cleavage-resistant mutation in mice will enable studies to explore the biological relevance of MERTK shedding in vivo.

TAM RTKs were determined to have sequence similarity to the insulin receptor subfamily based on the intracellular tyrosine kinase subdomain/segment VII sequence $Y(N_3)YYY$. However, due to overall dissimilarities, they were placed in a unique subfamily as opposed to being considered part of the insulin receptor subfamily. The TAM RTK kinase domain is

most closely related to *Ron* and *Met* (14). TAM RTKs are characterized by a signature KWIAIES sequence that distinguishes them from other RTKs. Three major tyrosine autophosphorylation sites—Tyr^{749}, Tyr^{753}, and Tyr^{754}—have been identified in MERTK (54). These tyrosine residues are conserved in AXL and TYRO3 and have been identified by proteomic approaches to be frequently phosphorylated (55), yet their requirement for the kinase activity of these receptors remains to be tested. Also within their intracellular domains, AXL and MERTK but not TYRO3 carry a conserved ITIM (immunoreceptor tyrosine-based inhibitory motif, LL**Y**SRL) (56). All three receptors have ITIM-like sequences (E(I/M)**Y**(D/N)**Y**L) that are most similar to those found in the PIR-B immunoreceptor (57). Tyrosine phosphorylation of ITIMs in PIR-B leads to the recruitment and activation of the protein tyrosine phosphatase SHP-1 and the negative regulation of various immune cell types. Whether a similar mechanism is engaged upon TAM receptor activation remains to be explored.

Although the domain organization of the TAM RTKs and ligands has been well characterized (Figure 1), the nature of the receptor–ligand complexes in various cell types and their functional significance remain to be fully investigated. The individual affinities of GAS6 and PROS1 toward TYRO3, AXL, and MERTK are known: GAS6 can bind all three TAM receptors with higher affinities for AXL and TYRO3 in comparison to MERTK (58), whereas PROS1 can activate TYRO3 and MERTK (28, 59) but not AXL. Nevertheless, it remains unknown if the TAM receptors and ligands can exist as homo- or heterodimers and what the affinities of the ligands GAS6 and PROS1 are for the individual receptor assortments. Finally, the role of PtdSer binding in the overall conformation of the ligand-receptor complex and how it affects receptor activation is not well understood. Further characterization of the biochemical composition and the structure of these ligand-receptor complexes in the presence and absence of phospholipids is required for an improved understanding of TAM RTKs.

FUNCTION

TAM RTK signaling functions to restore homeostasis by distinct, yet integrated, mechanisms: This pathway negatively regulates inflammation by limiting the intensity and, perhaps, the duration of the immune response. TAM RTKs also function in phagocytosis, specifically the phagocytic removal of apoptotic cells and debris. Such a cleanup act is necessary to prevent continuous inflammation, and it signals a change from “attack-the-pathogen” mode to “repair-and-restore tissue/organ function” mode. Consistent with these roles, TAM RTKs are expressed primarily by myeloid cells of the immune system (Tables 1 and 2). Additionally, TAM RTKs function in platelet stabilization and vascular integrity, as yet another wound-healing and repair process aimed at restoring the predamage state.

Taming Inflammation

Innate immune cells such as dendritic cells (DCs) and macrophages recognize pathogens through their pattern-recognition receptors, including the Toll-like receptors (TLRs) (1). A variety of cytokines and chemokines are released by innate immune cells to recruit specialized cells and cause local inflammation. DCs and macrophages also engulf certain pathogens and present foreign antigens to T helper cells to engage the adaptive immune

response (60). The adaptive response is shaped by the prevalent cytokine milieu, and a specific, antigen-directed response ensues. This crescendo of collective activity between innate and adaptive immunity culminates in a robust antipathogen response. It is imperative for the organism that inflammation is reined in once the adaptive immune system has been engaged. Although essential for triggering and shaping adaptive immunity, the persistence of innate immune responses can cause substantial harm and collateral damage, as these are not antigen-restricted. At this juncture, the necessity of negative regulatory mechanisms becomes apparent, for there is a fine line between a successful versus a rogue response.

The adaptive immune system, once activated, is capable of negatively regulating its own level of activation as well as dampening innate immunity through multiple mechanisms. Co-inhibitory cell surface receptors present on T cells, such as CTLA-4 and PD-1, are induced upon T cell activation and can attenuate the immune response (7–9). The most well-studied pathway is the dampening of TCR signaling through the activation of tyrosine phosphatases. Other negative regulators, such as the cytokine IL-10, function as general suppressors of T cell, DC, and macrophage function (61).

TAM RTKs and TAM ligands are essential negative regulators of the immune system and function at the interface of innate and adaptive immunity (62). This mechanism is primed through upregulation of TAM RTK expression upon activation of DCs (63, 64). Nonetheless, the TAM pathway is engaged only after the ligands become available, which happens once the adaptive immune response has been initiated. TAM signaling acts specifically on the innate immune system to disengage it from further antigen presentation and cytokine production. In this way, TAM RTKs and their cognate ligands act as antigen-specific brakes of the innate immune system (62). Below, we describe the historical sequence of the discoveries that led to our current understanding of TAM function in immune regulation.

Tyro3, *Axl*, and *Mertk* were originally discovered by Lai & Lemke (13) during their effort to identify novel RTKs involved in early development and organization of the nervous system. Lai, Lemke, and colleagues later determined that *Tyro3* was strongly expressed in the brain, a finding consistent with a potential function in neural development (20). The first indication that these RTKs may have important immune function came from observations made by Camenisch et al. in 1999 (65). These authors generated $Mertk^{-/-}$ mice in an effort to identify the physiological function of this RTK. MERTK is expressed in macrophages, and when peritoneal exudates from $Mertk^{-/-}$ mice were cultured in vitro, LPS treatment resulted in significantly increased TNF-α levels in comparison to cultures from wild-type mice (65). Importantly, in vivo the LD_{50} of LPS for $Mertk^{-/-}$ mice was half of that for wild-type mice. $Mertk^{-/-}$ mice had about threefold enhanced serum TNF-α when administered 100 mg/kg of LPS, and ~90% of mice succumbed to endotoxic shock (65). In 2001, Lu & Lemke (57) further characterized mice that were triple knockout for the TAM receptors ($Tyro3^{-/-}$ $Axl^{-/-}$ $Mertk^{-/-}$ or TAM RTK TKO). To their surprise, these TAM RTK TKO animals did not present with serious developmental anomalies as expected and appeared superficially normal (57). Even the immune system was apparently normal at birth, as no differences were noted in the size of the secondary lymphoid organs or in the numbers of cells of myeloid or lymphoid lineages.

As the TAM RTK TKO mice matured, they developed chronic inflammation and systemic autoimmunity. At about 4–6 weeks after birth, a remarkable difference in the size of spleen and lymph nodes between TAM RTK TKO and wild-type mice was observed (57). Although some features of autoimmunity were detectable by four weeks of age, a stronger phenotype was noticeable at 6–8 months. TAM RTK TKO mice, especially the females, had thromboses and hemorrhages in many tissues, including the brain. Footpads and joints were swollen, and lesions were visible on the skin. These mice had high levels of antibodies to dsDNA, collagens, and phospholipids, such as cardiolipin, PtdSer, phosphatidylethanolamine, and phosphatidylinositol. Histological analyses revealed IgG deposits in kidney glomeruli (57). The TAM RTK TKO phenotype is reminiscent of features of autoimmune diseases in humans including systemic lupus erythematosus (SLE), pemphigus vulgaris, psoriasis, and rheumatoid arthritis.

The abnormal growth of the secondary lymphoid organs in TAM RTK TKO mice and the chronic inflammatory and autoimmune phenotype can be ascribed to hyperproliferation of B and T cells. Yet, somewhat enigmatically, the TAM RTKs were not readily detected in B and T cells. Furthermore, in vivo transfer experiments in which spleen cells from wild-type mice were transferred to TAM RTK TKO mice indicated that the lymphoid hyperproliferation phenotype was non–cell autonomous (57). As described above, TAM RTKs are expressed in antigen-presenting cells (APCs), such as DCs and macrophages (Tables 1 and 2). APCs from TAM RTK TKO mice showed increased response to various TLR agonists and enhanced production of type I interferons (IFNs) (63). Additionally, MHC class II, CD86, and IL-12 levels were significantly higher in TAM RTK TKO–derived APCs when compared to wild-type (63). Therefore, the splenomegaly and lymphadenopathy observed in TAM RTK TKO mice can be ascribed to the initiation by APCs of the hyperactivation of B and T cells.

How do TAM RTKs function? In vitro experiments provided direct evidence that TAM RTKs are significantly upregulated in DCs as a consequence of TLR engagement. Axl mRNA was induced by type I IFNs produced downstream of TLR activation (63, 64, 66). Thus, this braking mechanism is not available at the onset of the immune response, but manifests only following initiation and acceleration of the immune response. This may be a safeguard so that the brake cannot be employed mistakenly. A conceivable exception may be tolerogenic environments, where the brake might always be engaged to prevent initiation of the immune response. However, TAM RTK function in these environments remains to be investigated.

The mechanism of TAM RTK action involves upregulation of the suppressor of cytokine signaling proteins SOCS1 and SOCS3 (63). Consistent with a central role for TAM RTK in the negative regulation of inflammation, the upregulation of *Socs* by type I IFNs was contingent on TAM RTK. SOCS1 induction by IFN-α was significantly reduced in TAM RTK TKO DCs (63). These SOCS E3 ubiquitin ligases are responsible for pleiotropic downregulation of the immune response through the turnover of molecules that function in critical, positive regulatory signaling cascades, such as TLR, NF-κB, and JAK-STAT pathways (67). Substrates of SOCS1 and SOCS3 include MAL, TRAFs, and JAKs. Not only did TAM RTK signaling shut down the TLR and type I IFN receptor-JAK-STAT signaling–

dependent upregulation of proinflammatory molecules, but it did so by hijacking elements of this pathway into functioning as key negative regulatory components. For example, the TAM RTK–dependent upregulation of *Socs* required the type I IFN receptor and also STAT1 (63). Therefore, the very same receptor and transcription factor that drive the initial proinflammatory response were usurped to keep inflammation from going berserk. Although the mechanism is not entirely clear at present, evidence indicates that TAM RTKs can complex with type I IFN receptors and modify STAT function (63), potentially by altered phosphorylation. Such a switching mechanism would likely ensure a rapid inhibition of inflammation, with the ubiquitin ligase–mediated degradation of adaptors ensuring fail-safe termination. In conclusion, TLR/type I IFN signaling and TAM RTK signaling illustrate a paradigm of interconnectedness of opposites—TLR/type I IFN signaling enables inflammation and leads to the upregulation of TAM RTKs, and TAM RTK signaling halts inflammation by disengaging TLR/type I IFN signaling (63). An additional mechanism of TAM RTK–mediated inhibition of inflammation includes the upregulation of the transcription factor *twist*, which in turn leads to downregulation of TNF-α (64).

The aforementioned experiments revealed how the braking mechanism functions, but how the brake is engaged in the context of a physiological immune response has been described only recently. Recombinant TAM ligands GAS6 and PROS1 potently suppressed the activation of DCs and consequent cytokine production triggered by engagement of TLR3, -4, or -9 in vitro (63). Therefore, one or both of these ligands were expected to be the in vivo mechanism of engaging the TAM braking machinery. One attractive mechanism, wherein the innate immune response is allowed to gear up and set in motion the adaptive immune response unhindered, i.e., without being subject to negative regulation, would involve the adaptive immune system itself applying the brake to halt inflammation. Indeed, we have recently demonstrated that PROS1 is expressed in activated, but not resting, T cells (62). Both human and mouse T cells, once activated by in vitro antigen presentation or in vivo immunization, displayed PROS1 on their cell surface (62). In fact, activated T cells had already been described as a source of PROS1 by Smiley et al. in 1997 (68). However, at that time, the function of TAM RTKs in immune regulation was not known. Hence, the primary function of T cell–derived PROS1 was thought to be related to its anticoagulant activity.

Functional evidence for T cell–derived PROS1 as a physiologically relevant TAM ligand came from the genetic knockout of PROS1. The complete genetic ablation of PROS1 was lethal (35). However, when PROS1 was selectively deleted in T cells, we found that T cell–derived PROS1 suppressed APC activation and limited cytokine production in an antigen-specific, TAM RTK–dependent manner (62). In vivo experiments confirmed that T cell–specific PROS1 knockout mice had a significantly heightened immune response following immunization (62). Low levels of PtdSer are transiently exposed on the outer leaflet of the plasma membrane in live $CD4^+CD45RB^{lo}$ activated/memory cells as well as in activated $CD8^+$ T cells (69, 70). Consistent with the requirement of PROS1 to bind to PtdSer for bioactivity, competitive inhibition of PtdSer binding impaired PROS1 anti-inflammatory function. It is possible that PROS1 is secreted by activated T cells and subsequently binds surface-exposed PtdSer. However, it is interesting to speculate that PROS1 is already bound to PtdSer on the luminal membrane of the endoplasmic reticulum. Increased exocytosis in

activated T cells could therefore lead to the enrichment of PtdSer-bound PROS1 on T cell surfaces. Taken together, these results indicate that PROS1 produced by activated T cells acts on the TAM RTKs in DCs and restrains DC activation and cytokine production. Furthermore, this mechanism is conserved in humans, given that antibody-mediated blockade of human PROS1 in mixed lymphocyte reactions resulted in increased activation of human DCs (62). Thus, PROS1–TAM RTK signaling functions at the interface of adaptive and innate immunity and is perfectly poised to limit the intensity and, perhaps, the duration of the immune response to prevent collateral damage associated with runaway inflammation (Figure 2). Intriguingly, other immune cells such as B cells also expose PtdSer on their outer membrane leaflet (71). Therefore, TAM ligand production by adaptive immune cells for the engagement of TAM RTKs in innate immune cells may be a general principle for maintaining immune homeostasis.

Phagocytosis of Apoptotic Cells

In 1908, the Nobel Prize in Physiology or Medicine recognized Metchnikoff for his discovery of phagocytosis as an important immune defense mechanism by which specialized cells engulf and kill pathogens. A select form of phagocytosis is the clearance of dead cells and cellular debris, a process termed efferocytosis (72). This is a monumental task that removes $>10^9$ apoptotic cells per day in humans. The vast majority of apoptotic cells are generated as part of the normal cellular turnover. For example, under homeostatic conditions, immune cells such as neutrophils have an extremely short half-life. Once neutrophils become apoptotic, they are promptly cleared by macrophages and DCs. Efferocytosis of neutrophils under homeostatic conditions regulates granulopoiesis (73).

Upon inflammation and hypoxia, the half-life of neutrophils that infiltrate the tissue is extended, favoring the accumulation of neutrophils at the affected site. Once the trigger of inflammation is eradicated, an active mechanism enables the resolution of the inflammatory response (11). Hallmarks of resolution and tissue repair include inhibition of neutrophil influx and clearance of the apoptotic neutrophils (11).

TAM receptors have been shown to mediate the efferocytosis of apoptotic neutrophils generated as a consequence of the inflammatory response (74) (Figure 3). Bosurgi et al. (74) observed that *Axl*$^{-/-}$ *Mertk*$^{-/-}$ mice accumulate increased numbers of TUNEL$^+$ Ly6G$^+$ neutrophils in the lamina propria of the large intestine after dextran sodium sulfate (DSS)-induced inflammation. The total number of live neutrophils in the lamina propria was similar between *Axl*$^{-/-}$ *Mertk*$^{-/-}$ and wild-type mice before and after DSS treatment, indicating that the defect was specifically in the clearance of apoptotic neutrophils upon inflammation (74). Interestingly, this study came to the conclusion that loss of AXL and MERTK in the radiosensitive hematopoietic compartment did not contribute to this phenotype, given that chimeric mice receiving *Axl*$^{-/-}$ *Mertk*$^{-/-}$ bone marrow progenitors did not have defects in clearance of TUNEL$^+$ Ly6G$^+$ cells. *Axl*$^{-/-}$ *Mertk*$^{-/-}$ mice also had significantly enhanced colonic inflammation, as evidenced by colonoscopic score, colon length, histopathological examination, and proinflammatory cytokine production by lamina propria leukocytes (74). Again, the loss of AXL and MERTK in the radiosensitive hematopoietic compartment did not contribute to this phenotype, but rather the phenotype

depended on an AXL^+ $MERTK^+$ radioresistant, tissue-resident population of macrophages. Interestingly, the ImmGen Consortium identified MERTK as one of a panel of markers expressed universally on mature, tissue-resident murine macrophage populations, including peritoneal macrophages, red pulp macrophages of the spleen, lung macrophages, and microglia (75). Using an unbiased approach, Xue et al. (76) recently identified MERTK as one of five cell surface markers that allow the distinction of human macrophages from DCs and monocytes.

Previously, MERTK was demonstrated to be an important receptor on macrophages that mediated the clearance of apoptotic cells. When dexamethasone was injected into mice to induce death of cortical thymocytes, *Mertk*$^{-/-}$ mice showed a sevenfold increased accumulation of apoptotic thymocytes (77). The rate of cell death itself was unaltered in *Mertk*$^{-/-}$ mice. Furthermore, MERTK was not expressed in thymocytes. The authors also directly examined the clearance of apoptotic cells. Peritoneal macrophages were stimulated with thioglycollate, and fluorescently labeled apoptotic thymocytes were injected into the peritoneal cavity. Alternatively, fluorescently labeled syngeneic apoptotic lymphocytes were injected intravenously, and clearance was measured within the spleen. Irradiated *Mertk*$^{-/-}$ mice reconstituted with wild-type bone marrow showed clearance of dexamethasone-induced apoptotic thymocytes at near-normal levels (77). Interestingly, the converse experiment in which *Mertk*$^{-/-}$ bone marrow was transferred into irradiated wild-type mice demonstrated normal removal of apoptotic cells. This was ascribed to compensation by radioresistant wild-type macrophages. In vitro experiments confirmed that both *Mertk*$^{-/-}$ and wild-type macrophages bound apoptotic thymocytes similarly. However, *Mertk*$^{-/-}$ macrophages had a dramatic deficit in phagocytosis of apoptotic thymocytes but not of *Listeria*, latex beads, or opsonized particles (77).

Not only MERTK but also AXL and TYRO3 function in the phagocytosis of apoptotic cells. *Axl*$^{-/-}$, *Tyro3*$^{-/-}$, and *Axl*$^{-/-}$ *Mer*$^{-/-}$ macrophages had ∼40–50% reduction in their abilities to phagocytose apoptotic thymocytes (78). However, the relative contribution of the three TAM RTK family members in the removal of apoptotic cells may be unique to the cell type of the phagocyte. For example, bone marrow–derived DCs from *Mertk*$^{-/-}$ mice did not demonstrate a significant reduction in phagocytosis of apoptotic thymocytes, whereas *Axl*$^{-/-}$, *Tyro3*$^{-/-}$, and *Axl*$^{-/-}$ *Mer*$^{-/-}$ mice all had severe deficits in this process (78).

Autoimmune diseases such as SLE have been associated with an accumulation of apoptotic debris. Consistent with a role for MERTK in the clearance of apoptotic cells and with the accumulation of apoptotic debris in SLE, *Mertk*$^{-/-}$ mice display a lupus-like disease (79). Therefore, two aspects of TAM RTK function described above—i.e., the inhibition of TLR signaling as well as the phagocytic removal of apoptotic cells—are associated with the prevention of autoimmunity and the maintenance of tissue homeostasis. Specific phosphorylation sites in MERTK may be critical in inducing the distinct downstream activities. Tibrewal et al. (80) identified a MERTK mutation, Y867F, that failed to stimulate actin cytoskeleton reorganization and phagocytosis but retained the ability to dampen TLR signaling. $MERTK^{Y867F}$ failed to induce FAK Y^{861} phosphorylation and tyrosine phosphorylation on $p130^{CAS}$. Yet the ability to inhibit LPS-stimulated NF-κB activation was retained by this MERTK mutant (80). The causal contribution of these two individual

functions of MERTK in preventing autoimmunity remains to be fully dissected. These functions may be independent but most likely are integrated. MERTK-dependent phagocytosis of apoptotic cells led to suppression of the inflammatory response by preventing NF-κB activation and cytokine production (81). LPS stimulation of bone marrow–derived DCs led to the activation of IκB kinase (IKK), degradation of IκB, and activation of NF-κB. Preincubation of DCs with apoptotic thymocytes, before LPS administration, resulted in the failure of IKK and NF-κB activation. This effect required MERTK, as the use of *Mertk*$^{-/-}$ DCs or blocking antibodies against MERTK in the assay failed to show inhibition of NF-κB activation (81). Cross talk or feedback between distinct signaling cascades downstream of MERTK regulating cytokine production and phagocytosis may exist and have not been comprehensively characterized.

The TAM RTK–TAM ligand interaction functions as a bridge between apoptotic cells and the phagocytes. Apoptosis is perhaps the most well-known biological context in which PtdSer is exposed on the outer leaflet of the plasma membrane. During apoptosis, the Ca^{2+}-independent scramblase XKR8 is activated by caspase-mediated cleavage (82). GAS6 directly binds PtdSer but not phosphatidylcholine, phosphatidylethanolamine, or phosphatidylinositol (83). Furthermore, this interaction facilitated GAS6 binding to AXL by reducing the K_d by ~30%. As discussed above, PROS1, with serum concentrations of 300 nM, also binds PtdSer. Anderson et al. (42) discovered that the presence of serum enhanced the phagocytosis of apoptotic cells but not of necrotic cells by macrophages in vitro. Biochemical fractionation experiments, experiments with purified proteins, and immunodepletion approaches have all identified the activity in the serum as PROS1. The authors also demonstrated that PROS1 binds apoptotic cells in a Ca^{2+}-dependent manner. Whereas the Gla domains of GAS6 and PROS1 bind the PtdSer exposed on the outer leaflet of the apoptotic cell plasma membrane, their laminin G1 domains bind TAM RTKs (45, 49). Notably, TAM RTKs are commonly coexpressed and cooperate with other phagocytic receptors that recognize PtdSer either directly or indirectly through their ligands. Two such receptors are TIM-4, a direct PtdSer receptor, and αvβ5 integrin, which binds MFG-E8 that, in turn, recognizes PtdSer (84, 85).

Although we have focused on the role of TAM signaling in the phagocytosis of apoptotic cells by cells of the immune system, this pathway is also known to mediate clearance of apoptotic cells or cell fragments by a variety of other cell types. The locus responsible for inherited retinal dystrophy in the Royal College of Surgeons (RCS) rat, a classical model of inherited blindness, was mapped to *Mertk* by positional cloning (86, 87). In the eye, pigmental epithelial cells phagocytose rod outer segments diurnally. RCS retinal pigmental epithelial cells failed to phagocytose rod outer segments in culture conditions, indicating that failure to remove shed rod outer segments was the cellular basis of this condition (88). Patients with recessively inherited retinopathies harbor mutations in the *MERTK* locus that result in predicted loss or reduction in MERTK function (89). Similarly, TAM RTKs in Sertoli cells are instrumental for phagocytosis of apoptotic germ cells in the testis and TAM RTK TKO male mice are sterile (90, 91). MERTK in astrocytes appears to mediate the phagocytosis and elimination of synapses (92). We direct the reader to additional reviews

for an in-depth description of MERTK-dependent phagocytosis by nonimmune cells (93, 94).

Vascular Integrity

Consistent with the function of TAM signaling in the regulation of the immune response to injury and promotion of tissue repair, TAM RTKs and ligands have been shown to participate in vascular homeostasis. Vascular damage results in the exposure of subendothelial collagen and von Willebrand factor, leading to the adhesion and initial activation of platelets (95). In parallel with platelet adhesion, the exposure of blood contents to tissue factor expressed by subendothelial smooth muscle cells and adventitial fibroblasts triggers the rapid initiation of the extrinsic coagulation cascade. Multiple positive feedback mechanisms between platelet and clotting activation ensure the efficient sealing of the wound. For example, PtdSer exposure on the surface of activated platelets serves as the docking site for GLA-containing coagulation factors, whereas generation of thrombin as a result of coagulation promotes platelet aggregation through the protease-activated receptors PAR1 and PAR4. Furthermore, activated platelets produce a plethora of factors that induce endothelial cell survival, proliferation, and vascular repair. TAM RTK signaling has been shown to promote the stabilization of platelet aggregation, survival of endothelial cells, and restitution of endothelial barrier function, contributing overall to the prompt wound-healing response of the damaged vasculature.

Genetic knockout of TAM receptors results in increased blood loss upon tail clipping in mice (96). Angelillo-Scherrer and colleagues (96, 97) were the first to ascribe this phenotype to the function of TAM RTKs and GAS6 in the stabilization of thrombus formation (Figure 3). The aggregation of platelets at the site of injury proceeds in a two-step manner centered on the $\alpha_{IIb}\beta_3$ platelet integrin. The initial platelet activation triggers pathways within the cell that cause a conformational change in $\alpha_{IIb}\beta_3$ integrin at the cell surface. This conformational change increases the affinity of this integrin for its ligand fibrinogen. This is known as inside-out signaling. Reduced platelet stabilization in TAM RTK TKO mice was not due to deficient inside-out signaling, as binding of fibrinogen to $\alpha_{IIb}\beta_3$ was normal (96). Next, irreversible platelet aggregation is mediated by classical outside-in signaling events. Fibrinogen–$\alpha_{IIb}\beta_3$ integrin interaction at the cell surface leads to tyrosine phosphorylation in the cytoplasmic region of the β_3 subunit of $\alpha_{IIb}\beta_3$. This step allows myosin binding and clot retraction. It is in this second phase that TAM RTKs and GAS6 have been reported to function. When *Tyro3*$^{-/-}$, *Axl*$^{-/-}$, and *Mertk*$^{-/-}$ platelets were stimulated in vitro with low concentrations of platelet agonists, such as ADP, collagen, or thromboxane A2 analogs, they failed to aggregate efficiently (96). Higher concentrations of platelet agonists were able to override the aggregation deficit of TAM RTK TKO platelets, indicating that the TAM axis potentiates platelet aggregation at low doses but is redundant at higher doses of platelet agonists. In fact, defective aggregation in TAM RTK TKO mice was due to impaired amplification of outside-in $\alpha_{IIb}\beta_3$ signaling. Recombinant GAS6 was found to induce tyrosine phosphorylation of the β_3 subunit of $\alpha_{IIb}\beta_3$ in wild-type platelets, but it failed to do so in TAM RTK single knockout platelets. The ability of Gas6 to promote phosphorylation of $\alpha_{IIb}\beta_3$ correlated with enhanced activity of PI3K and AKT, a signaling axis known to mediate the strength of platelet aggregation (96). A similar requirement of TAM RTKs and

GAS6 was described for the stabilization of platelet aggregation in humans. Recombinant human GAS6 potentiated the phosphorylation of AKT and the aggregation of human platelets induced by ADP (98). Notably, although the TAM RTK pathway potentiates the response to ADP, activation of TAM RTKs alone is unable to promote platelet aggregation.

Angiogenesis is a critical component of wound healing, and TAM signaling has been shown to participate in the formation of new vessels. *Axl*$^{-/-}$ mice showed increased vascular permeability to Evans blue dye injected in the tail vein (35). AXL is expressed in endothelial cells and was found to mediate their proliferation and arrangement into capillary-like structures or tubes in vitro (99, 100). Interestingly, AXL was required for VEGF-A-induced migration of endothelial cells and neovascularization (99). The role of AXL in angiogenesis was also reported in the neovascularization of tumors (101, 102). AXL function in angiogenesis might not be restricted to endothelial cells. Smooth muscle cells express the TAM receptor AXL, and these cells stabilize the nascent blood vessels. Activation of AXL in vascular smooth muscle cells prevented apoptosis in serum-starved conditions (103). Furthermore, AXL was expressed in vascular smooth muscle cells in the neointima after balloon catheter injury in the rat carotid (104). Therefore, cell type–specific ablation of *Axl* would be required to ascribe this phenotype to the loss of AXL expression in endothelial, vascular smooth muscle, and/or immune cells.

The TAM agonists GAS6 and PROS1 are also expressed by endothelial cells. Indeed, their high expression in conditioned media from the endothelial cell line ABAE allowed Stitt and colleagues (28) to discover these proteins as factors that bind and activate TAM RTKs. PROS1 was also found to be expressed in murine and human endothelial cells (35, 105). Interestingly, although PROS1 in the plasma was mostly thought to be produced by hepatocytes, Burstyn-Cohen and colleagues (35) used a conditional knockout approach in which *Pros1* was deleted in *Tie2*-expressing cells to show that approximately 50% of PROS1 in circulation originates from endothelial/hematopoietic cells.

Both ligands have been reported to contribute to vessel integrity. GAS6 promotes cell survival of endothelial and smooth muscle cells upon growth arrest (40, 106, 107). Furthermore, GAS6 stimulates the proliferation, migration, and tube formation of human retinal endothelial cells (108). The most well-characterized function of PROS1 in hemostasis is as an anticoagulant. However, similar to the other major anticoagulant, aPC (109), PROS1 also mediates direct cytoprotective effects on vascular cells. The cytoprotective function of both aPC and PROS1 appears to be mediated by their ability to activate the endothelial Protein C receptor or the TAM receptors, respectively, rather than by their roles in the clotting cascade. PROS1 was reported to enhance the barrier function of endothelial cells in vitro. When human brain endothelial cells were cultured in a monolayer and exposed to a hypoxic/ischemic insult, the addition of human PROS1 to the culture was able to prevent the disruption of the barrier (110). The protective function of PROS1 was mediated by TYRO3, as the effect was ablated when *TYRO3* was silenced in human brain endothelial cells or when *Tyro3*$^{-/-}$ murine brain endothelial cells were tested. PROS1 has also been shown to act as a potent mitogen of vascular smooth muscle cells in vitro (41, 111).

A clear mechanistic understanding of PROS1 contribution to hemostasis in vivo is complicated by its dual functions as an anticoagulant and as a TAM agonist. *Pros1*$^{+/-}$ mice showed increased vascular permeability of Evans blue (35). Is the defective vascular integrity in *Pros1*$^{+/-}$ mice secondary to increased thrombosis and perturbed blood flow during vascular development or a direct consequence of the loss of Pros1–TAM RTK signaling in vascular cells, or both? Genetic approaches that disable PROS1 function as a TAM agonist while preserving its anticoagulant role and vice versa should enable the molecular dissection of PROS1 function in hemostasis. Finally, it is intriguing to hypothesize that the function of the TAM axis in platelet stabilization, endothelial, and smooth muscle cell biology forms part of a coordinated response that together with TAM function in inflammation and efferocytosis instructs the repair of the injured tissue.

TAMs IN DISEASES

Autoimmune and Chronic Inflammatory Diseases

The TAM pathway has been implicated in various human chronic inflammatory and autoimmune diseases, including multiple sclerosis (MS), SLE, inflammatory bowel diseases, and rheumatoid arthritis. MS is a demyelinating autoimmune disease (112). Although the autoantigen(s) in MS remain unknown, the autoimmune response targets the myelin sheath and oligodendrocytes, cells that are responsible for myelination of neurons in the central nervous system. The first studies to suggest a role for TAM signaling in this disease came from mouse models of demyelination using the toxin cuprizone. When cuprizone is provided in the mouse chow, it induces the death of oligodendrocytes and demyelination of the corpus callosum. Once cuprizone is withdrawn, remyelination ensues. Genetic ablation of AXL or GAS6 in mice led to a significant increase in demyelination after cuprizone administration (113, 114). These mice also showed a delay in the clearance of apoptotic debris and remyelination during the recovery phase (113, 115). Interestingly, the exogenous administration of GAS6 into the brain of wild-type mice had a therapeutic effect and enhanced the remyelination after cuprizone-induced injury (116). Furthermore, the protective effect of AXL signaling was not limited to the cuprizone model; it was also described in the experimental autoimmune encephalitis model in which mice are immunized with a peptide from the myelin oligodendrocyte glycoprotein. In this model, a more severe disease was observed in *Axl*$^{-/-}$ mice than in control mice (117). The impact of the loss of function of the other TAM members—TYRO3, MERTK, and PROS1—in murine models of demyelination remains to be explored. And evidence of a role for TAMs in demyelinating diseases is not limited to mouse studies. A large genome-wide association study including more than 9,000 cases of MS identified an association between polymorphisms in *MERTK* and susceptibility to this disease (118). A similar association was also reported by an independent study (119). Interestingly, a recent expression quantitative trait locus study revealed an association between the risk alleles in *MERTK* for MS and a reduced expression of this RTK in peripheral blood human monocytes (120).

The cell type in which the loss of TAM RTK signaling is key for the development of demyelinating diseases remains unknown. Two TAM-dependent functions appear to be relevant in demyelinating diseases. First, TAM receptors are expressed in microglia (Tables

1 and 2) (114, 121, 122), a cell type that plays a central role in the response to injury in the CNS. Analogous to that described in DCs and macrophages, TAM signaling mediates an anti-inflammatory effect in microglia (114, 123). An increased number of activated IBA1^{+} microglia were detected in the corpus callosum of *Gas6*$^{-/-}$ mice upon administration of cuprizone (114). Second, TAM RTKs are expressed in oligodendrocytes, and GAS6 has been shown to promote the survival of oligodendrocytes upon exposure to TNF-α in vitro and cuprizone treatment (115, 116, 124). Given the influence of the inflammatory response of microglia on the survival of oligodendrocytes and vice versa, selective ablation of TAM components in each of these cellular compartments would be needed to more clearly dissect the role of TAM signaling in mouse models of demyelination/remyelination.

As described above in the section on “Taming Inflammation,” a role for TAM signaling has also been proposed in SLE. A hallmark of this disease is the production of autoantibodies against self-antigens such as DNA. During B cell activation in the lymph node, any autoreactive B cell that is generated as a consequence of somatic hypermutation usually dies by apoptosis and is rapidly cleared. A type of macrophage, known as the tingible body macrophage (TBM), is responsible for removing these apoptotic B cells. Prompt phagocytosis is fundamental for avoiding the activation of any neighboring autoreactive B cells and subsequent autoimmunity (125). MERTK has been shown to be expressed by TBMs (Tables 1 and 2) (126). Furthermore, the genetic ablation of MERTK in mice led to the accumulation of apoptotic cells in the germinal center (GC) of lymph nodes and to a lupus-like autoimmune disease (79, 126, 127). Although the etiology of SLE remains unknown, it is interesting to note that apoptotic debris is present in GCs from SLE patients, a rare event in healthy individuals (128).

Deficiencies in the TAM ligand PROS1 have also been frequently detected in SLE patients (129–131). PROS1 deficiencies can occur because of germline mutations (132–143) (Figure 4). These deficiencies are classified based on the concentration and anticoagulant function of PROS1 in the blood. For example, type I deficiencies result from reduced production of a functional (anticoagulant) PROS1. Type II deficiencies are characterized by normal levels of PROS1, but the protein is defective in its anticoagulant function. Type III deficiencies are due to reduced amounts of free PROS1 (not bound to C4BP) but normal amounts of total PROS1. It is interesting to note that although mutations that lead to type I and type III deficiencies can be found everywhere in the gene, those associated with defective anticoagulant function (type II) appear to cluster at the 5′ end of the gene (Figure 4). Nongenetic causes of PROS1 deficiencies include pregnancy, oral contraceptives, antibodies against PROS1, hepatic failure, and vitamin K recycling inhibitors such as warfarin. Similarly, PROS1 deficiencies associate with other autoimmune and chronic inflammatory diseases, such as Crohn’s disease and ulcerative colitis (144–146).

Given the discovery of the anti-inflammatory, prophagocytic, and cytoprotective functions of PROS1 via the TAM RTKs, it remains to be established if the loss of these individual functions in patients with PROS1 deficiencies contributes to the onset and progression of chronic inflammatory and autoimmune diseases. None of the known PROS1 deficiencies to date have been classified based on these additional functional properties of PROS1. Intriguingly, thromboembolic events have been reported as comorbidities in SLE and

inflammatory bowel diseases (147). In light of the discovery of the dual function of PROS1 as anticoagulant as well as TAM RTK agonist, it is tempting to speculate that this combination of functions provides a unifying theory for this gene as a causal factor in autoimmune diseases associated with a procoagulant state. It is important to remember that PROS1 deficiency has not yet been established as a causal mutation for autoimmune diseases. Therefore, it remains possible that deficiencies in PROS1 merely allow a coincidental superposition of a prothrombotic state onto inflammation of unknown etiology. Furthermore, thrombosis can also lead to inflammation (148), and thus the reduced function of PROS1 as an anticoagulant alone might directly favor not only a procoagulant state but also a proinflammatory environment. Dissecting the anticoagulant versus the TAM RTK–dependent function of PROS1, both through identification of naturally occurring mutations and through experimental means in mouse models, will be required to fully understand the contribution of PROS1 in these diseases.

In light of the ability of the TAM pathway to temper inflammation, this signaling axis has become an attractive target for therapeutic interventions based on the delivery of TAM agonists. As described above, delivery of recombinant GAS6 was effective in a mouse model of demyelination (116). A recent study also showed that the intra-articular adenoviral delivery of TAM agonists reduced joint pathology in a murine model of rheumatoid arthritis (149). Interestingly, glucocorticoids, which are used broadly in the treatment of autoimmune and chronic inflammatory diseases, as well as agonists for the nuclear receptors PPARδ and LXR, have been reported to induce TAM components (Tables 1 and 2), and investigators have suggested that their tolerogenic effects are in part driven by the TAM RTK pathway (150, 151). Improved understanding of the specific immunological function of the TAM RTKs and their agonists is likely to pave the way for tailored therapeutic approaches in chronic inflammatory and autoimmune diseases.

Infectious Diseases

TAM RTKs and their ligands have been described as affecting viral infectivity. Kawaoka's laboratory (152) expressed a cDNA library from the filovirus-susceptible Vero cells in Jurkat cells, a human T cell line resistant to *Filoviridae*, to identify genes that could confer susceptibility to pseudotype viruses carrying Zaire Ebola or Marburg glycoproteins. This unbiased screen revealed that AXL favors filovirus infections. Furthermore, expression of TYRO3 and MERTK were similarly able to confer susceptibility to Ebola and Marburg viruses (152). This raised the possibility that TAM RTKs could play a specific role in filovirus entry. However, it was soon found that AXL could favor the infectivity of a wide array of viruses: vaccinia (153), Lassa (154), dengue (155), and West Nile (59). The broad spectrum of viruses with disparate glycoproteins that could infect cell lines or DCs in culture in a TAM RTK–dependent manner suggested that these RTKs did not function as specific receptors regulating tropism or viral entry (156). What could be the unifying principle behind this role for TAM RTKs in viral infection?

A common element in all the viruses described above is the exposure of PtdSer on the viral envelope. Viruses, as well as certain protozoa, employ a strategy to evade the immune system known as apoptotic mimicry, wherein they expose PtdSer on their outer surface

(157). Various studies revealed that the TAM ligands GAS6 and PROS1 as well as their binding to PtdSer were required for mediating TAM-dependent viral infection (59, 153, 155). Furthermore, enveloped viruses potentiated the activation (phosphorylation) of TAM receptors by their cognate ligands, and viral infection was dependent on TAM receptor kinase activity. The expression of kinase dead receptors or the addition of a small molecule TAM RTK inhibitor to the cell culture reduced the capacity of TAM RTKs to favor viral infection in vitro (59, 155, 158). It is interesting to note that although most of the studies have reported the ability of enveloped viruses to hijack the TAM pathway through PtdSer exposed on their surface, a recent study has shown that the major capsid protein of SV40, a nonenveloped virus, bears structural similarity to the TAM ligands and directly binds to the TAM receptors (159).

Thus, in the evolutionary arms race between host and pathogens, the fundamental role of TAM RTKs as negative regulators of the immune response appears to have been exploited by viruses to dampen and bypass the host defense. The activation of the TAM RTK downstream signaling pathway during infection with enveloped viruses is consistent with this hypothesis. When wild-type DCs were exposed to pseudotype viruses in vitro, the expression of negative regulators *Socs1* and *Socs3* was induced, only low levels of type I IFNs were detected, and the DCs were susceptible to infection. In marked contrast, TAM RTK TKO DCs failed to induce *Socs1* and *Socs3* and sustained a robust antiviral state that rendered them resistant to viral infection (59) (Figure 5, *left*).

Although multiple studies have concurred on the ability of TAM RTKs to favor viral infection in vitro, the function of this axis in viral infections in vivo remains controversial. Tabas's laboratory showed that *Axl*$^{-/-}$ mice were in fact more susceptible to herpes simplex virus (HSV)-1 infection in vivo (160). This phenotype was ascribed to the reduced capacity of *Axl*$^{-/-}$ DCs to cross-present viral antigens. Efferocytosis of infected apoptotic cells and cross-presentation of viral antigens are important mechanisms for priming cytotoxic T cell responses (161). In agreement with the function of AXL in the clearance of apoptotic cells (78), *Axl*$^{-/-}$ DCs were defective in cross-presenting OVA that was loaded onto apoptotic cells to CD8^{+} OT-I T cells in vitro. Furthermore, genetic ablation of AXL resulted in a significant reduction in proliferating HSV-1-specific CD8^{+} T cells upon HSV-1 infection in mice (160) (Figure 5, *right*). In marked contrast, using a different viral infection model, Hogaboam's laboratory showed the protective effect of an antibody against AXL during influenza infection (162). When wild-type mice were infected with a lethal dose of the PR8 influenza virus strain, the systemic administration of an anti-AXL antibody—reported to be an AXL antagonist—significantly reduced mice mortality. This protective effect correlated with increased expression of type I IFN and reduced lung pathology. Similarly, treatment with this anti-AXL antibody reduced the lung pathology upon respiratory syncytial virus infection in mice (162). Finally, Oldstone's lab failed to detect any significant differences in the response to lymphocytic choriomeningitis virus infection between wild-type and *Axl*$^{-/-}$ mice (163). In conclusion, the role of TAM RTKs in viral infections may be much more complex than their role in dampening type I IFN signaling.

Cancer

The discovery of TAM RTKs was spurred by two major thematic interests: their putative role in development and differentiation, and their potential function in transformation and carcinogenesis. For example, not only was *Tyro3* identified in Schwann cells, murine stem cells, fetal mouse brain, human brain, ovary, and testis and speculated to function in development, but this RTK was also found in human teratocarcinoma, mouse mammary tumor cells, a chemically transformed human breast cell line, and H-*ras* and v-*erbB* transduced mouse embryonic fibroblasts (164). The transduction of *Tyro3* enabled colony formation of rat fibroblasts in soft agar (20, 164). *AXL* was independently cloned as a transforming oncogene from chronic myelogenous leukemia patients and leukemia cell lines (21, 22), and *AXL*-transduced NIH/3T3 could form foci in culture and tumors in the nude mouse. *Mertk* was recognized as an oncogene based on its similarity to v-*Ryk* (25), and the human ortholog was cloned from a B-lymphoblastoid expression library (165). It is expressed during mouse development, especially in the monocytic lineage of hematopoietic cells. Ectopic expression of MERTK in lymphocytes and thymocytes using a transgenic approach resulted in T cell lymphoblastic leukemia/lymphoma phenotypes in mice (166).

The predominant literature on TAM signaling in cancer focuses on its cell autonomous oncogenic function in tumor cells (for a detailed account, see Reference 167). Currently, the consensus indicates that therapeutic targeting of TAM RTKs will prove efficacious in cancer. A number of small molecule kinase inhibitors and biologics targeting TAM RTKs are being developed and tested in preclinical models (101, 102). Most notably, BerGenBio recently completed a Phase Ia trial. The alternative body of literature reveals TAM function in tumor-associated immune cells. Here, the verdict is more ambiguous, as both pro-oncogenic and anti-oncogenic roles have been described. How do these parallel strains of TAM function within tumor cells and in tumor-associated immune cells determine the final outcome in the context of an intact organism? As with the lives of *Weronika* and *Véronique* in Krzysztof Kieślowski's *La Double Vie de Véronique*, are these functions interconnected or irreducibly disparate?

One of the first evidences of a TAM signaling axis involving tumor cells and tumor-associated macrophages came from the experiments of Loges et al. (168). These authors demonstrated that tumor-infiltrating macrophages display higher levels of Gas6 than do their splenic counterparts (168). This raises the possibility that factors predominant or enriched in the tumor microenvironment, such as IL-10 and M-CSF, lead to GAS6 upregulation in tumor-associated macrophages (168). Tumor-associated macrophages, in turn, use the upregulated Gas6 to engage TAM receptors in tumor cells (Figure 6a). This TAM signaling nexus promotes tumor cell proliferation. Whereas this study was based on mouse models, another study recently showed that human bone marrow from acute myeloid leukemia (AML) patients, including AML mononucleated cells and $CD34^+$ $CD38^-$ AML stem cells, has higher levels of AXL (169). Correspondingly, AML bone marrow–derived stromal cells expressed higher levels of GAS6 compared to normal bone marrow–derived stromal cells (169). The finding that stromal-derived GAS6 can fuel the proliferation of AML cancer cells was validated by in vitro coculture experiments using human AML cell lines and GAS6-expressing stromal cells.

GAS6 induction in cells of the tumor microenvironment has also been reported in the context of prostate cancer. Hematopoietic stem cells bind Annexin II expressed on the surface of osteoblasts to home to the endosteal niche. Prostate cancer cells use an identical mechanism to home to the bone marrow. The interaction of Annexin II with the Annexin II receptor led to the production of GAS6 in osteoblasts and AXL expression in tumor cells (170). Surprisingly, GAS6-AXL signaling inhibited the proliferation of PC3 and DU145 prostate cancer cell lines in a dose-dependent manner (170). Therefore, this signaling pathway induced dormancy, inhibited apoptosis, and limited chemotherapy-induced cell death in this model.

Whereas the last two examples describe how stromal cells engage TAM RTK signaling in tumor cells, a non–tumor cell autonomous function of TAM RTKs in tumor growth and progression has also been described. Cook et al. (171) reported that *Mertk* ablation in the MMTV-PyMT (mouse mammary tumor virus LTR–driven expression of polyoma virus middle T antigen) mouse model of breast cancer or syngeneic transplantation of melanoma and colon cancer cells in $Mertk^{-/-}$ mice resulted in reduced tumor growth and metastasis. Resistance to tumor growth was conferred by bone marrow transplantation from $Mertk^{-/-}$ to wild-type mice, indicating that MERTK function in a radiosensitive hematopoietic compartment aids tumor growth and metastasis. The absence of MERTK resulted in reduced IL-10 and increased IL-12 and IL-6 levels in the tumor microenvironment, suggesting that MERTK functions to dampen antitumor immune responses. Indeed, immunodepletion of $CD8^+$ T cells in $Mertk^{-/-}$ mice led to increased tumor growth (171) (Figure 6a).

Consistent with this idea of a protumorigenic, non–tumor cell autonomous role for TAM RTK, a recent study ascribed the therapeutic efficacy of a potent small molecule inhibitor of TAM RTKs in reducing cancer metastasis in mice to the inhibition of TAM RTK signaling in NK cells and the subsequent enhancement of NK cell activation and their antimetastatic activity (172). Although the molecular mechanism for TAM RTK–mediated inhibition of NK function remains unknown, it is intriguing to hypothesize that it depends on the ITIM-like sequences in TAM RTKs, a feature that these receptors share with the inhibitory NK cell receptors (57). However, germline deletion of TAM RTKs led to impaired differentiation of NK cells (173). Therefore, further studies using mouse models with inducible ablation of TAM RTKs are required to dissect the function of these receptors in NK cell differentiation versus activation.

Two other reports, in contrast, describe an anti-oncogenic role for TAM signaling in tumor-associated immune cells. Interestingly, both these reports use a model of inflammation-induced colon carcinogenesis. In humans, colitis-associated cancer is a subtype of colorectal cancer. Patients with chronic intestinal inflammation and inflammatory bowel diseases are at a significantly increased risk of colorectal cancer. Similarly, in mice, a single dose of the procarcinogen azoxymethane, which is metabolized into its active form by the gut microbiota, followed by repeated dosing with the proinflammatory agent dextran sodium sulfate in the drinking water (AOM-DSS treatment), results in the induction of colon cancer. $Gas6^{-/-}$ mice showed increased susceptibility to AOM-DSS treatment in comparison to wild-type mice (174). $Gas6^{-/-}$ mice displayed a significantly greater number of PCNA- and c-Myc-positive polyps, higher levels of the proinflammatory cytokine TNF-α and the

chemokines CXCL1 and CCL2, increased NF-κB activation, and reduced survival. Another type of colorectal cancer in humans involves germline loss of *APC* (*Adenomatous Polyposis Coli*). In the analogous mouse model of colon cancer driven by mutations in the *Apc* loci (Apc^{Min}), the loss of Gas6 similarly increased disease susceptibility (174).

An independent study from our laboratory also came to a similar conclusion regarding TAM signaling in colonic inflammation and colon cancer. $Axl^{-/-}$ $Mer^{-/-}$ mice had more numerous, larger polyps and increased colonoscopic tumor score after AOM-DSS treatment in comparison to wild-type mice (74). Consistent with tumor incidence, $Axl^{-/-}$ $Mer^{-/-}$ mice exhibit an exaggerated response to DSS. DSS, when administered in the absence of AOM, causes colonic inflammation. Under this treatment regimen, $Axl^{-/-}$ $Mer^{-/-}$ mice underwent severe loss of body weight, and colonoscopy revealed increased granularity, loss of vasculature and translucency, and looser stool consistency. Postmortem histological analyses revealed increased ulcerations, crypt hyperplasia, crypt loss, leukocyte infiltration, and edema. Consistent with the dual function of TAM RTKs in the inhibition of innate immune signaling and the phagocytosis of apoptotic cells, $Axl^{-/-}$ $Mer^{-/-}$ colons exhibited an increased number of apoptotic $Ly6G^{+}$ neutrophils and increased IFN-γ and TNF-α production (74). $Axl^{-/-}$ $Mer^{-/-}$ lamina propria macrophages responded to the inflammatory trigger by producing increased amounts of proinflammatory mediators such as iNOS and TNF-α, whereas they failed to produce adequate amounts of tissue repair factors such as RELMα, IL-10, and TGF-β (74) (Figure 6b). Therefore, TAM signaling in colonic inflammation and colorectal cancers is consistent with an anti-inflammatory and antitumor function.

In summary, there appears to be somewhat of a paradox that belies a consensus regarding the outcome of inhibiting TAM RTK in cancer. The functional effect of TAM signaling during immune cell–cancer cell interaction may vary with the tumor type. The stage of the tumor—early stage, such as carcinogenesis and tumor initiation, versus late stage, such as tumor progression and metastasis—may also be crucial in determining how TAM function in tumor-associated immune cells can influence therapeutic outcomes. Various types of immune cells infiltrate the tumor microenvironment and can either favor or oppose tumor growth depending on their identity. TAM signaling in different types of immune cells needs to be comprehensively catalogued. Even in mouse models of cancer, the use of TAM inhibitors versus the complete absence of TAM RTKs from birth can alter the interpretation of TAM RTK function in cancer. Additional studies investigating TAM signaling function in various malignancies are imperative to resolve this apparent paradox. This is particularly important as the blockade of immune checkpoints, such as anti-CTLA-4 and anti-PD-1 therapies, is emerging as an effective strategy in the fight against cancer. Thus, evidence-based targeting of the TAM RTKs may complement existing immunotherapy regimens to unleash the full power of the anticancer immune response.

CONCLUSION

Akin to the theological pursuit of singularity in nondualistic traditions, inflammation and TAM signaling constitute a mechanism of binary opposition—polar forces that work in concert—to enable a physiological immune response (Figure 7). The knowledge that TAM

signaling is a negative regulator participating in dampening inflammation reveals the importance of curbs and constraints for a physiological immune response. In conjunction with immunoregulation, TAM signaling in efferocytosis and in vascular integrity establishes this pathway as a fundamental element in the return to baseline and restoration of tissue/organ function after an effective antipathogen response.

Acknowledgments

We apologize to those whose work we were unable to cite owing to space limitations. We thank Edward Mandell for modeling the domains of TAM RTKs and ligands presented in Figure 1 and acknowledge Cynthia Orensztajn for the illustration in Figure 7. This work was supported by grants from the National Institutes of Health (R01AI089824 to C.V.R. and S.G., CA95060 to S.G.) and by the Crohn's and Colitis Foundation (to C.V.R and S.G.), the American Heart Association (C.V.R), American Asthma Foundation (C.V.R.), Lupus Research Institute (C.V.R), and American Italian Cancer Foundation Postdoctoral Research Fellowship (L.B.).

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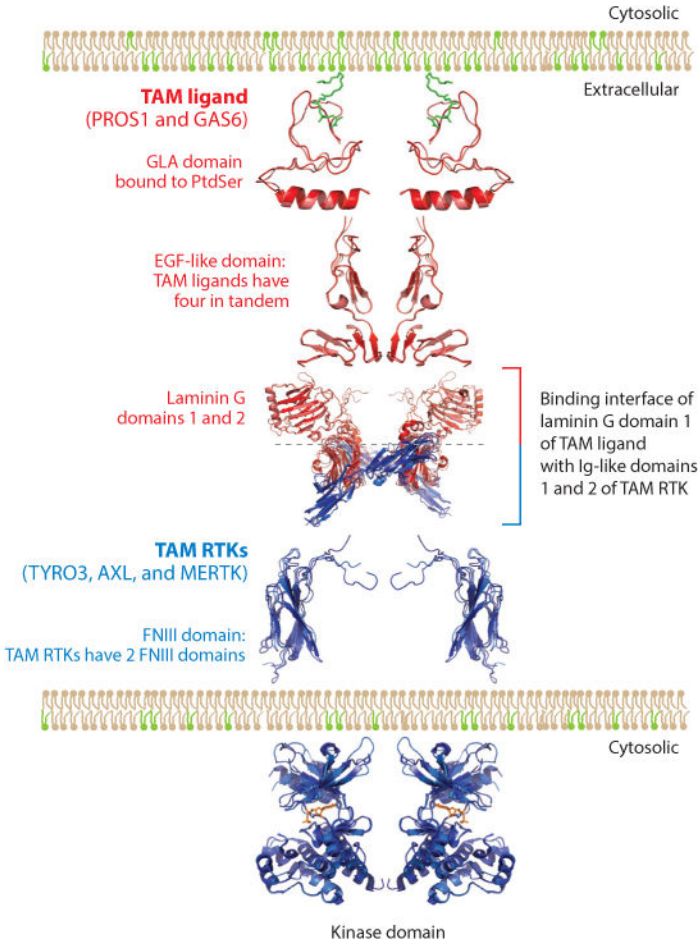

Figure 1.

Domain organization of TAM receptors and ligands. The TAM ligands GAS6 and PROS1 (*red*) carry an N-terminal GLA domain that binds to PtdSer (*green*) exposed on the plasma membrane of cells in different settings, including apoptosis, immune activation, and coagulation. GLA domains are followed by four EGF-like repeats. At the C terminus, the TAM ligands carry two laminin G domains, of which domain 1 interacts with the Ig-like domains of the TAM RTKs to form a heterotetrameric complex. Ig-like domains are followed by two FNIII domains and an intracellular tyrosine kinase domain (*blue*). The domain structures show superimposed sequences of the three TAM receptors or two ligands and were predicted from either crystallographic data or sequence homology. The structure of the whole complex—including PtdSer, TAM ligands, and TAM receptors—remains unknown. (Abbreviations: EGF, epidermal growth factor; FNIII, fibronectin type III; RTK, receptor tyrosine kinase; TAM, TYRO3, AXL, and MERTK.)

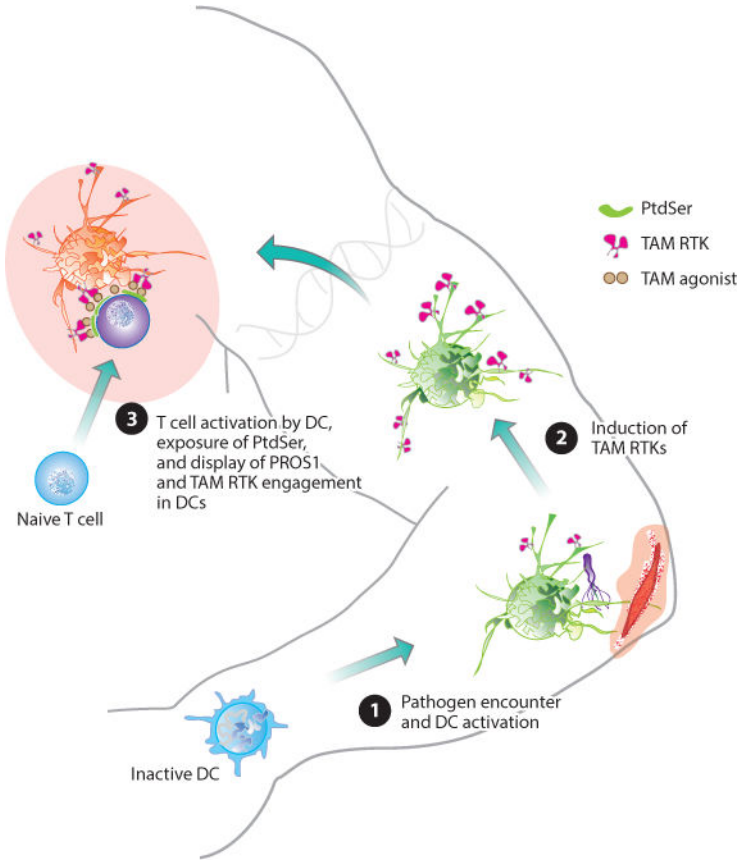

Figure 2.

TAM signaling functions as a homeostatic negative feedback mechanism of the immune response. Patrolling dendritic cells (DCs) are activated upon pathogen encounter, leading to DC maturation ❶, migration to the draining lymph node, and induction of TAM RTK expression in DCs ❷. When DCs reach the draining lymph node, they present antigens to T cells, triggering T cell activation, exposure of PtdSer on the surface of activated T cells, and display of the TAM agonist PROS1. Once activated, T cell–derived PROS1 reports back to the DC through the inhibitory TAM RTKs in DCs ❸.

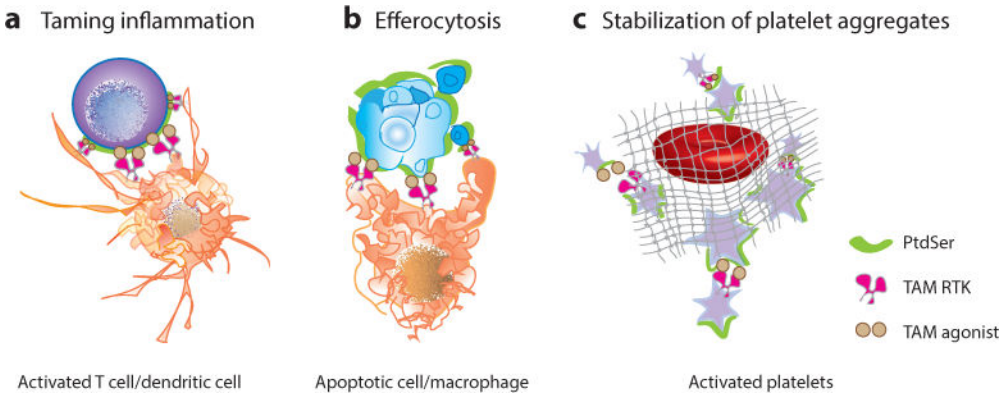

Figure 3.

A triad of TAM functions in the homeostasis of the immune response. (*a*) TAM RTK signaling is engaged at the T cell–dendritic cell interface, whereby activated T cells expose PtdSer on their surface and express the TAM agonist PROS1 to activate the TAM RTKs in dendritic cells and regulate the magnitude of the immune response. (*b*) TAM RTK signaling mediates the phagocytosis of apoptotic cells, which is also known as efferocytosis. Apoptotic cells and membranes expose PtdSer on their surfaces. Binding of PtdSer by the TAM agonists triggers the activation of TAM RTKs in phagocytes such as macrophages. (*c*) TAM RTK signaling promotes the stabilization of platelet aggregates upon vascular injury. Activated platelets expose PtdSer on their surface that functions as a platform for the binding of various GLA-containing proteins, including the TAM agonists. Platelets also express the TAM RTKs, and activation of these receptors potentiates platelet aggregation and clot retraction. TAM RTK signaling also functions in endothelial and vascular smooth muscle cells (see text) to promote wound healing of the damaged vasculature.

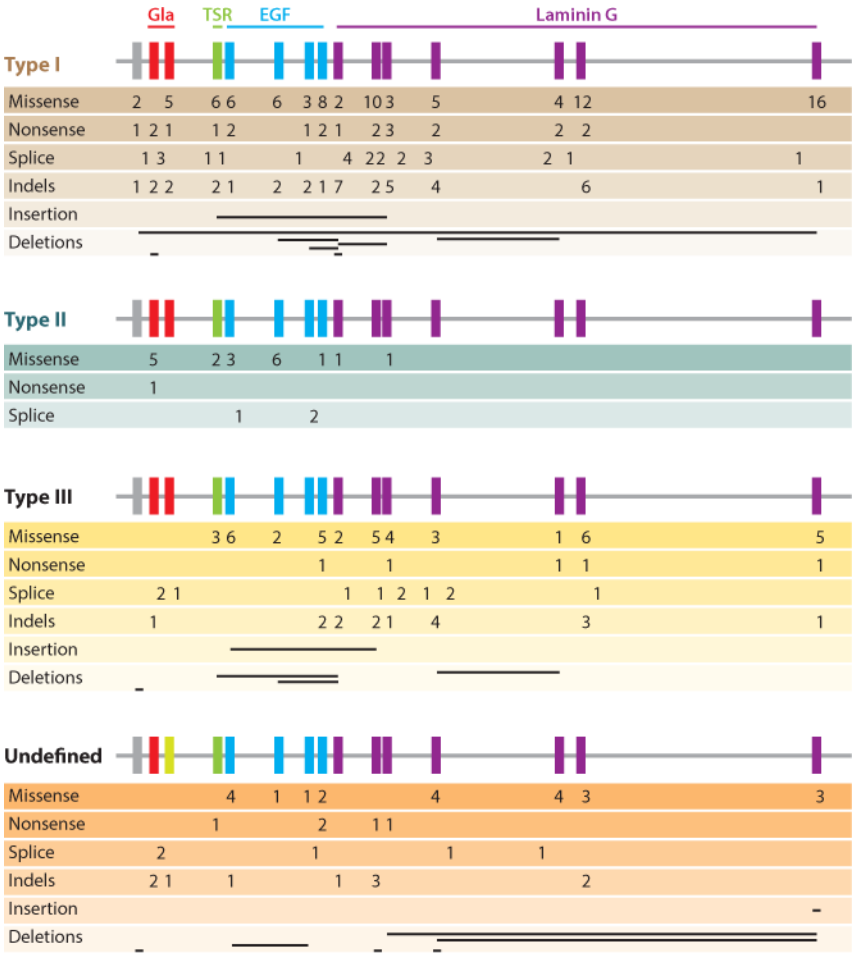

Figure 4.

Schematic representation of *PROS1* mutations, with the frequency of mutations in specific introns and exons of the *PROS1* gene subdivided according to the type of PROS1 deficiency. Those mutations in *PROS1* for which available clinical data are not sufficient for their classification into type I/II or III deficiency are presented as undefined. (Abbreviations: EGF, epidermal growth factor; TSR, thrombin-sensitive region.)

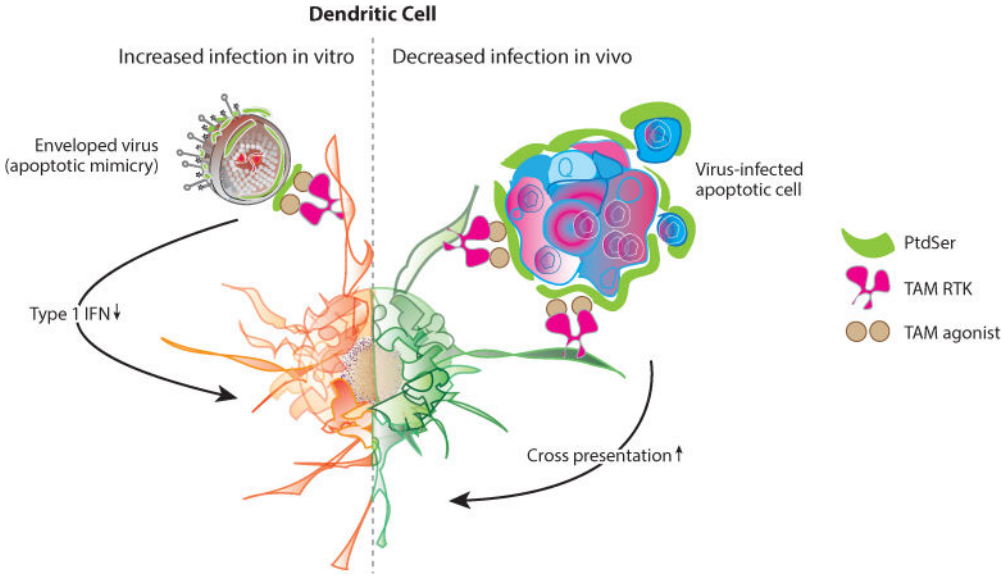

Figure 5.

Contrasting functions of TAM signaling in viral infection. Enveloped viruses employ apoptotic mimicry, or the exposure of PtdSer on the viral envelope, to hijack the TAM pathway in dendritic cells and promote infection. Exposed PtdSer on the viral envelope potentiates the activation of TAM RTKs by the TAM agonists, leading to the suppression of the antiviral type I IFN response and favoring the infection of dendritic cells in vitro. Whether a similar mechanism occurs in vivo remains ill-defined (*left*). In contrast, TAM RTK signaling can favor the cross-presentation of viral antigens and lead to decreased viral infection in vivo. Phagocytosis of virally infected apoptotic cells in a TAM RTK–dependent manner leads to cross-presentation and the induction of a protective antiviral adaptive immune response (*right*).

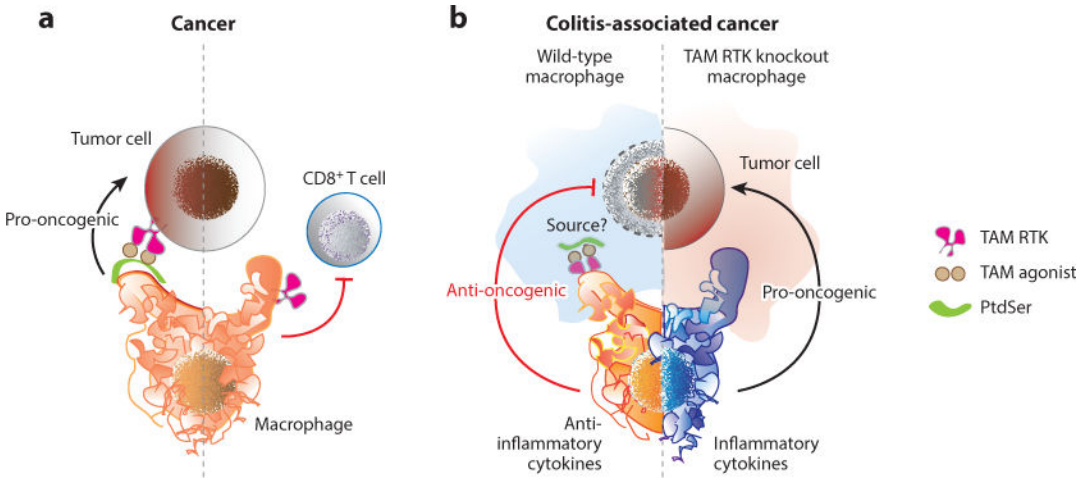

Figure 6.

Immunological functions of TAM signaling at the tumor-stroma interface. (*a*) TAM signaling can favor tumor growth in cancer through two independent mechanisms. Tumor-associated macrophages express the TAM agonist GAS6 and promote tumor growth through the activation of oncogenic TAM signaling in tumor cells (*left*). Activation of MERTK in tumor-associated macrophages leads to an immunosuppressive cytokine environment, decreased antitumor $CD8^+$ T cell responses, and increased tumor growth. (*b*) In contrast, in colitis-associated cancer, TAM RTK signaling in intestinal lamina propria macrophages promotes an anti-inflammatory environment that limits chronic inflammation and associated tumors. The absence of this RTK pathway favors a pro-inflammatory environment in the colon and an increased incidence of colitis-associated cancer.

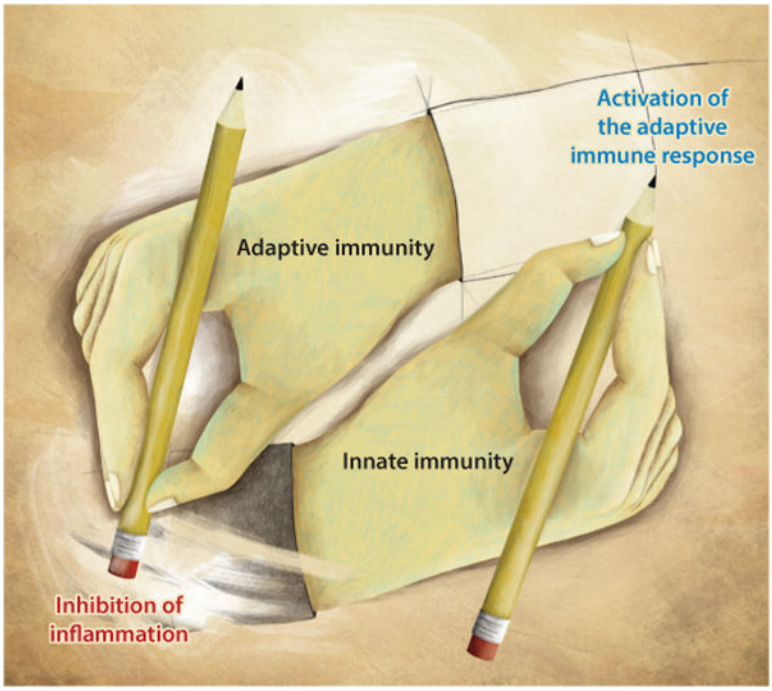

Figure 7.

The TAMing of inflammation (with apologies to William Shakespeare). The illustration depicts the intimate interactions at the interface of the innate and adaptive immune responses, where antigen-presenting cells of the innate immune arm render the adaptive immune response. Upon activation, adaptive immune cells erase or limit the innate immune response.

Table 1

Cells of the murine immune system that express TAM receptor tyrosine kinases and ligands

	TAM receptors			TAM ligands	
Murine cells	**TYRO3**	**AXL**	**MERTK**	**GAS6**	**PROS1**
Adipose tissue					
Macrophages F4/80$^+$ CD11c$^+$			FACS (75)		
Bone marrow derived					
DCs CD11c$^+$	FACS (78), WB (81)	FACS (63, 78), WB (63, 81, 160), qPCR (175), IF (160)	FACS (78, 176, 177), WB (63, 81, 177), qPCR (175)		PCR (78)
DCs CD11c$^+$ CD8α$^+$			FACS (177)		
DCs CD11c$^+$ B220$^+$			FACS (177)		
DCs CD11c$^+$ stimulated with LPS, PolyI:C or IFN-α		FACS, qPCR, WB (63)			
DCs CD11c$^+$ stimulated with LXR ligands		qPCR (175)	qPCR (175)		
Macrophages CD11b$^+$ F4/80$^+$		Gene array (178, 179)	Gene array (178, 179), qPCR (179), WB (180)	Gene array (178), PCR (78), qPCR (179)	Gene array (178), PCR (78)
Macrophages CD11b$^+$ F4/80$^+$ stimulated with apoptotic cells			qPCR (179)	qPCR (179)	
NK cells	PCR (173)	PCR (173)	PCR (173)		PCR (173)
Blood					
Platelets	FACS (181), WB (97)	FACS (181), WB (97)	FACS (181), PCR (182)	WB (97)	
Brain					
Microglia CD11b$^+$ CD45^{lo} F4/80^{lo}			FACS, gene array (75)		
Microglia FCRLS$^+$ CD11b$^+$			Gene array, qPCR (122)	Gene array, qPCR (122)	Gene array, qPCR (122)
Microglia FCRLS$^+$ Ly6C$^-$ in EAE mouse model			Gene array (122)	Gene array (122)	
Microglia Iba-1$^+$	WB (121)	WB (121)	WB (121), IF (92)		
Microglia Iba-1$^+$ after cuprizone challenge in vivo		IHC (114)	IHC (114)		
Heart					
Macrophages CD11b$^+$ F4/80$^+$ CD206$^+$			FACS (183)		
Macrophages CD11b$^+$ F4/80$^+$ CD206$^+$ after AngII challenge			FACS (183)		

	TAM receptors			TAM ligands	
Murine cells	**TYRO3**	**AXL**	**MERTK**	**GAS6**	**PROS1**
Intestine					
Macrophages CD11b^{+}			Gene array (75)		
Macrophages CD11b^{+} F4/80^{hi} after DSS challenge in vivo		RT-PCR (74)	qPCR, FACS (74)		
Liver					
Macrophages F4/80^{+}CD11c^{int}			FACS (75)		
Lymph nodes					
T cells CD3^{+} activated with IL-4 in vitro					NB, WB (68)
OT-II transferred congenic T cells after OVA immunization					FACS (62)
Lung					
DCs CD11b^{+} CD103^{-} CD14^{hi}			FACS (75)		
Macrophages SiglecF^{+} CD11c^{+}			FACS, gene array (75)		
Macrophages SiglecF^{-} CD11c^{+}			FACS (75)		
Activated alveolar macrophages			FACS (184)		
Peritoneum					
Macrophages		qPCR, WB (185)	FACS (75, 176), gene array (75), qPCR (185), WB (185, 186)	ELISA, qPCR (168, 185)	ELISA, qPCR (185)
Thioglycollate-elicited macrophages	FACS (78)	FACS (78)	FACS (78, 176, 187), WB (53, 180), qPCR (180), gene array (75)		
Thioglycollate-elicited macrophages stimulated with LXR/RXR ligands			qPCR (175, 180), WB (180)		
Thioglycollate-elicited macrophages stimulated with aged neutrophils ex vivo		qPCR (175)	qPCR (175, 180), WB (180)	qPCR (175, 180)	
Spleen					
DCs CD11c^{hi}		FACS (160), WB (63)	IF (126, 187), WB (63), FACS (176)		
DCs CD11c^{hi} CD8α^{+}		FACS (160)	FACS (176)		
Macrophages CD11b^{+} FSCH^{high}/SSC-H^{high}			FACS (176, 188)		
Macrophages F4/80^{hi} B220^{-} CD11c^{+} MHCII^{+} red pulp			IF (187), FACS, gene array (75)		
Macrophages F4/80^{+} CD68^{+} (tingible body)			IF (126, 187)		

	TAM receptors			TAM ligands	
Murine cells	**TYRO3**	**AXL**	**MERTK**	**GAS6**	**PROS1**
Activated $CD4^+$ T cells					FACS, qPCR (62)
NK cells	FACS (172)	FACS (172)	FACS (172, 176)		
NKT cells			FACS (176)		
Platelets $CD61^+$			IF (187)		
Thymus					
Apoptotic thymocytes				FACS, WB (177)	
Tumor microenvironment					
DCs $CD11c^+$ (CT26 colon carcinoma)				qPCR (168)	
Macrophages $MAC3^+$ (CT26 colon carcinoma)				IHC, qPCR (168)	
Macrophages $Gr1^-$ $CD11b^{high}$ (CT26 colon carcinoma)				qPCR (168)	
Macrophages (MMTV-PyMT mammary tumor model)			FACS (189)		

Abbreviations: DC, dendritic cell; DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; FACS, fluorescent activated cell sorting; IF, immunofluorescence; IHC, immunohistochemistry; NB, Northern blot; NK, natural killer; PCR, reverse-transcription polymerase chain reaction; qPCR, real-time polymerase chain reaction; WB, Western blot.

Table 2

Cells of the human immune system that express TAM receptor tyrosine kinases and ligands

	TAM receptors			TAM ligands	
Human cells	**TYRO3**	**AXL**	**MERTK**	**GAS6**	**PROS1**
Blood					
Monocytes			FACS (190)		
Monocyte-derived DCs $CD1c^+$			FACS (190)		
Monocyte-derived DCs $CD11c^+$ differentiated with GM-CSF and IFN-α		FACS, qPCR (66)		qPCR (66)	
Monocyte-derived DCs $CD11c^+$ differentiated with GM-CSF and IFN-α, PolyI:C, TLR7/8, or CD40 ligand			FACS (66)		
Monocyte-derived DCs $CD11c^+$ stimulated with dexamethasone			FACS, qPCR (191)		
Monocyte-derived macrophages		Gene array (192)	Gene array, FACS (76), WB (53, 193)	Gene array (192)	
Monocyte-derived macrophages stimulated with dexamethasone			Gene array (76, 192)		
Monocyte-derived macrophages stimulated with IFN-α		qPCR (64)			
Activated $CD4^+$ T cells					FACS, qPCR (62)
Basophil/mast cells stimulated with IFN-α		PCR (194)			
$CD34^+$ HPCs in steady state and after stimulation with IL-15		FACS, PCR (195), WB (196)	PCR (195)	FACS, PCR (195)	
Platelets	FACS (181), PCR (97)	FACS (181), PCR (97), WB, electron microscopy (98)	FACS (181), PCR (97, 182), WB (53)	FACS, PCR (97), electron microscopy (98)	PCR (182)
Brain					
Microglia			qPCR (122)	qPCR (122)	qPCR (122)
Lymph nodes					
$CD4^+$ T cells from reactive lymph nodes					IHC (62)
Lung					
Alveolar macrophages			Gene array, RT-PCR, WB, FACS, IHC (197)		
Spleen					
DCs $CD141^+$		FACS (160)			

Abbreviations: DC, dendritic cell; FACS, fluorescent activated cell sorting; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPC, hematopoietic progenitor cell; IF, immunofluorescence; IFN, interferon; IHC, immunohistochemistry; NB, Northern blot; PCR, reverse-transcription polymerase chain reaction; qPCR, real-time polymerase chain reaction; WB, Western blot.